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EFFECTIVENESS OF THE REVACCINATION OF HAMADRYAS BABOONS WITH DRIED LIVE PLAGUE VACCINE NIIS AND YERSINIA PESTIS FRACTION I

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In order to prevent illnesses in plague-endemic regions /74* the USSR is using a live vaccine made on the basis of the EV plague microbe strain. Numerous studies have demonstrated that among the mass methods of plague vaccine immunization, the inhalation method has the greatest productivity, simplicity and is highly effective [1, 4]. At the same time it is known that a single injection of live vaccine, including by inhalation, does not ensure the formation of a lengthy and highly intensive immunity [5]. It is therefore necessary to experimentally develop an effective vaccination system that would guarantee a high level of resistance for a lengthy time in those inoculated.

We believe that a promising trend in solving this problem is use for revaccination of the method of application of live vaccine which differs from the method used in the primary inoculation. The experimental substantiation to this approach is the results of studies which demonstrated that microbes of the vaccine strain injected into immune animals by the same method as the primary inoculation were accepted in the body significantly worse, and therefore a pronounced revaccination effect was not attained [2]. Another method of improving the level of specific resistance could be boosting by protective antigens of the plague microbe. Insofar as manifestation of the revaccinating activit[illegible]

*Numbers in margin indicate pagination in original foreign text.

with the acceptance effect on which the action of live vaccines is based, it was interesting to use antigen preparations to study fraction I and the lipopolysaccharide (LPS) Yersinia pestis. Fraction I that was first isolated by Baker, et al. [7] in the opinion of the majority of researchers is the main immunogen of the plague microbe that is suitable both for primary immunization [6, 8] and for revaccination of laboratory animals that have been primarily inoculated with live vaccine [2]. The immunobiological properties of LPS of the plague pathogen have been characterized less completely, but this preparation was not previously used as a revaccinating agent. Because the bacterial endotoxins or the artificially produced analogs of the cellular wall components of both gram-negative and gram-positive microorganisms have pronounced adjuvant properties, it was important to assess the immunogenicity of a complex preparation consisting of fraction I and the plague microbe LPS.

The purpose of this work was to make a comparative study of the effectiveness of the NIIS live vaccine for needleless and inhalation methods of revaccination, as well as fraction I and its combination with LPS to revaccinate Hamadryas baboons against aerogenic infection with the plague pathogen.

Materials and Methods

A live dry plague vaccine NIIS prepared on the basis of the EV strain, fraction I produced from a deep culture of the vaccine strain EV microbes cultivated at 37°C, and LPS isolated by Davis [9] technique were used as the vaccine preparations.

The experiments were conducted on Hamadryas baboons weighing 3 - 8 kg. The animals were inoculated primarily by

aerosol of NIIS vaccine in a dose of 15 x 10^6 live microbes. The immunized animals were separated into five groups within 6 months. Monkeys of the first group were revaccinated by NIIS vaccine subcutaneously in a dose of 320 x 10^6 microbes, the second with the NIIS vaccine by inhalation (the aspiration dose was 15 x 10^6 microbes), the third by fraction I subcutaneously in a dose of 1mg on aluminum hydroxide gel, and the /75 fourth subcutaneously by a combination of fraction I (2 mg) and LPS (1 mg) on the same deposit r. Animals of the sixth group were not revaccinated (control II). Monkeys of the sixth group were vaccinated simultaneously with revaccination of the animals of the first to fourth groups (control I for assessing the activity of the booster dose of live vaccine with inhalation of 15 x 10^6 microbes). Preparations were injected subcutaneously using the BI-3 needleless injector into the inner surface of the femur. Within 6 months after revaccination the monkeys were infected by increasing doses of aerosol of plague pathogen.

TABLE 1. EFFECTIVENESS OF DIFFERENT IMMUNIZATION PLANS OF BABOONS WITH AEROSOL INFECTION

Group of Animals	Preparation Used for Revaccination	Preparation Dose	Method of Injecting Preparation	LD_{50}^{X}: K No. of Microbes	Resistance Index
First	NIIS	320 x 10^6 microbes	Subcutan.	13.75 x $10^{3}{}^{X}_{:}$ 2.50	47.8
Second	"	15 x 10^6 microbes	Inhalation	3.23 x $10^{3}{}^{X}_{:}$ 3.43	11.3
Third	Fraction I	2 mg	Subcutan.	25.13 x $10^{3}{}^{X}_{:}$ 3.95	87.5
Fourth	Fraction I + LPS	2 mg + 1 mg	"	3.08 x $10^{3}{}^{X}_{:}$ 2.03	10.7
Fifth (control I)	-	-	-	5.22 x $10^{3}{}^{X}_{:}$ 2.39	18.2
Sixth (control II)	-	-	-	1.69 x $10^{3}{}^{X}_{:}$ 2.10	5.9

(Table continued next page)

TABLE 1. Continued:

Seventh (control III-nonimmune animals)	-	-	-	0.29×10^3 ? 2.11 -

Note. Control I is determination of the booster dose of live vaccine activity with inhalation of 15×10^6 microbes, control II is assessment of the intensity of ground immunity within 12 months after inoculation with live vaccine by inhalation method (15×10^6 microbes).

The infection results were assessed by the LD_{50} index and the resistance index (J_{res}), the ratio of LD_{50} for immune animals to LD_{50} for intact ones. A pathological-anatomical and bacteriological study was made of the dead animals in order to establish the specific nature of infection. The degree of seroimmune reconstruction of the inoculated animals was assessed by the results of the reaction of passive hemagglutination with sheep erythrocytes sensitized by fraction I Y. pestis. The reliability of the differences between the averages was determined by the recommendations of G. F. Lakin [3].

Results and Discussion

The study results (Table 1) indicate a decrease in time of the immunity intensity in the baboons that were inoculated once by NIIS aerosol vaccine: the values I_{res} for the groups infected within 6 and 12 months after inoculation were respectively 18.2 and 5.9. Of the two revaccination methods (subcutaneous and inhalation) with live NIIS vaccine, subcutaneous was the most effective. Within 6 months immunity intensity in the animals revaccinated by the NII vaccination subcutaneously was over 4 times higher than with revaccination

by inhalation (for P = 0.95, the difference in the LD_{50} indicators was significant). The use of another method of application to stimulate immunity compared to primary inoculation apparently involves additional regional systems of immunity in the immune process, guaranteeing a more powerful antigen stimulation of the macroorganism and a corresponding increase in the specific resistance. Confirmation is the result of subcutaneous revaccination of the baboons with sorbed preparation of fraction I. Its subcutaneous injection into the baboons in a dose of 2 mg ensured relatively rapid accumulation in the blood sera of immunoglobulins against fraction I /76 (Table 2) and the best protection against aerosol infection of the plague pathogen compared to revaccination by inhalation of live vaccine. The difference between the live vaccine and fraction I during subcutaneous revaccination was unreliable with 0.95 probability.

TABLE 2. DYNAMICS OF ANTIBODIES AGAINST FRACTION I IN THE SERUM OF REVACCINATED BABOONS

Preparation Used for Revaccination	Method of Injecting Preparation	Mean Geometric Antibody Titer			
		Before Revaccination	In Different Periods After Revaccination		
			6th Day	26th Day	188th Day
NIIS vac.	Subcutan.	1:522 ± 3.22	1:652 ± 2.83	1:8473 ± 2.77	1:3093 ± 2.99
The same	Inhalation	1:480 ± 2.97	1:734 ± 2.90	1:10439 ± 2.87	1:1340 ± 3.23
Fraction I	Subcutan.	1:492 ± 3.40	1:12503 ± 3.98	1:44380 ± 3.80	1:3880 ± 4.63
Fraction I + LPS	"	1:2058 ± 3.08	1:27917 ± 2.70	1:55550 ± 2.83	1:3547 ± 2.94

Addition of the LPS of the plague microbe to the I preparation fraction thus did not increase the protective effect: the mean geometric titer of antibodies was approximately the

same as in the baboons that received a fraction of I and live vaccine subcutaneously. We believe that this experiment apparently used a surplus quantity of LPS preparation. As a result, additional injection of LPS did not foster the manifestation of the adjuvant effect, but on the contrary, suppressed the cellular link of immunity.

Conclusion

1. Subcutaneous revaccination by NIIS live plague vaccine guarantees the formation of a more intense immunity to aerosol infection by plague pathogen than the inhalation method of revaccination.

2. Revaccination by sorbed fraction of the I plague microbe in a dose of 2 mg did not differ in results from the subcutaneous method of revaccination by live plague vaccine.

3. The LPS Y. pestis in a dose of 1 mg combined with fraction I does not yield an adjuvant effect during revaccination.

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